Chapter 8

Summary, general discussion and future perspectives

SUMMARY

This thesis describes the delivery of apoptosis-inducing compounds to the Hepatic Stellate Cell (HSC) during liver fibrosis. HSC play a pivotal role in the fibrogenic process, which is a response to chronic liver injury. This cell starts to proliferate and accumulate within the injured liver in order to regulate tissue repair. During this activation process, increased amounts of extra cellular matrix components and inhibitors of matrix degradation are produced by these HSC and after chronic activation, the excessive matrix production will strongly affect the liver homeostasis [1,2]. Up till now, no effective therapy to treat liver fibrosis has reached the clinic, leaving liver transplantation as the only option left.

Importantly, animal model studies revealed that the fibrotic process is reversible. Rats with advanced liver fibrosis showed spontaneous resolution of fibrotic scar tissue after withdrawal of the damaging stimulus [3]. Even after established nodular cirrhosis, a near complete resolution of liver fibrosis was found after terminating the injury [4]. The resolution was accompanied by the loss of activated HSC via spontaneous apoptosis. This suggests that therapeutically inducing HSC apoptosis during fibrosis might accelerate restoration of the normal liver morphology [3,5,6].

However, the systemic use of apoptosis-inducing agents is likely to be associated with numerous side effects. Cell-selectivity of the drug is therefore crucial in this strategy. The specific delivery of appropriate drugs to the HSC may therefore be a relevant option for the treatment of liver fibrosis. Drug targeting technology largely alters the pharmacokinetic profile of a drug. If a proper homing deice is employed, it results in an accumulation of the drug at a specific site in the body, while limiting the adverse effects elsewhere.

In **chapter 1** the aim of the present thesis is presented. **Chapter 2** reviews the role of apoptosis in liver fibrosis. The pathophysiology of liver fibrosis is described and potential apoptosis-regulating drugs are discussed, including their safety concerns. It is argued that a possible way to overcome these problems may be to specifically target the selected drugs to HSC. This approach may lead to a novel treatment of liver fibrosis.

Recent publications show the ability of Gliotoxin, a fungal metabolite (GTX), to induce apoptosis in HSC. Not only in the carbon tetrachloride model, but also in the thioacetamide model of liver fibrosis, significant *in vivo* effects of GTX were found [7,8]. However, the effects of GTX on Kupffer and liver endothelial cells were not studied in any of these reports. Therefore, we examined this. **Chapter 3** describes the effect of GTX on not only the HSC, but on all cells in the liver assessed with liver slices. These slices, obtained from healthy and fibrotic rat livers showed a strong apoptotic response after incubation with GTX. Quantitative real-time PCR and

(immuno)histochemical staining revealed that all cells in the liver (HSC, Kupffer cells, endothelial cells and hepatocytes) were affected by GTX incubation. This emphasized the necessity to target GTX to the HSC. In **chapter 4** this targeted approach is explored. GTX was coupled to M6P-HSA, a HSC-selective carrier [9,10]. This GTX-M6P-HSA conjugate was able to induce apoptosis in cultures of human myofibroblasts and in fibrotic rat liver slices. In addition, this conjugate was able to bind specifically to rat HSC. The *in vivo* targeting experiment showed that GTX-M6P-HSA as well as untargeted GTX was able to reduce the expression of α-SMA, a marker for activated HSC, at the mRNA and the protein level. In addition, GTX affected hepatocyte functioning as reflected by a significant reduced PAS staining and increased serum AST (aspartate aminotransferase) levels. Hepatocytes rapidly respond to stress or injury by activation of glycogenolysis leading to a reduced glycogen content which can be demonstrated by PAS staining. An increase in serum AST levels is associated with hepatocytes damage [11]. Importantly, GTX-M6P-HSA did not affect these parameters, while clearly it reduced the expression of α-SMA, a marker for activated HSC. These results indicate that a targeted approach for GTX may provide a novel treatment strategy for liver fibrosis.

Prostaglandins are important mediators of inflammation. They tightly control inflammation responses and tissue repair mechanisms. The biosynthesis of prostaglandins is regulated by two cyclooxygenase (COX) enzymes. COX1 is constitutively expressed, whereas COX2 is highly inducible and expressed in many cells after cellular activation. COX-2 activity is observed in both Kupffer cells (KC) and Hepatic Stellate Cells (HSC) during liver fibrosis [12-15]. COX-2 activity plays a significant role in the control of several, sometimes opposite, processes during fibrosis, and only a cell-selective intervention may lead to the unravelment of the role of COX-2 in a particular cell type *in vivo.* In a study described in **Chapter 5** we explored the targeted delivery of the selective COX2 inhibitor niflumic acid (NFA) coupled to M6P-HSA. This study showed that the delivery NFA to HSC resulted in an increase in collagen type I and III deposition within the liver. These results implicate an important role for COX2-derived prostaglandins in the control of HSC activity during fibrogenesis.

One of the end products of the COX2-dependent biosynthesis of prostaglandins is 15-deoxy-$\Delta^{12,14}$-Prostaglandin J_2 (15d-PGJ_2). This cyclopentanone exhibits several effects including growth arrest, induction of apoptosis in different cell types and suppression of macrophage activation [16-18]. Recently, it was shown that this prostaglandin can induce apoptosis of human hepatic myofibroblasts (hMF) [19]. Sub-apoptotic concentrations of 15d-PGJ_2 strongly inhibited the proliferation of hMF and reduced the expression of interstitial collagens *in vitro* [20,21]. However, the broad and unspecific distribution profile of these lipophilic prostaglandins makes them

unsuitable for systemic application. They are not only taken up by many cells, rapidly metabolized in plasma, but also excreted in the urine, leading to a short half life in the bloodstream combined with a potential risk of adverse effects [22]. **Chapter 6** explores the effects of 15d-PGJ_2 coupled to the HSC-selective carrier M6P-HSA *in vitro* and *in vivo* in rats with liver fibrosis. The construct reduced proliferation and induced apoptosis in human hepatic myofibroblasts *in vitro.* In addition, the conjugate showed binding to isolated rat HSC and exhibited selective and rapid accumulation in the liver *in vivo*. Effect studies in fibrotic rats with 15d-PGJ_2 and 15d-PGJ_2-M6P-HSA showed a reduction in desmin-positive cells induced by both administered forms of 15d-PGJ_2, but only the conjugate was able to reduce procollagen 1α1 mRNA levels.

In recent years, three different HSC-selective carrier systems have been developed [9,23,24]. This thesis explores the use of two of them. The first system uses the sugar mannose-6-phosphate which is coupled to the protein human-serum-albumin to form M6P-HSA [9,10] and neoglycoprotein is taken up by the IGF-II/M6P-receptor expressed by HSC [25]. As indicated, **chapter 4, 5 and 6** outline the data obtained with this carrier.

The second HSC-delivery system contains small cyclic peptides that mimic the binding site of Platelet Derived Growth Factor BB to its PDGF-β receptor, chemically coupled to the protein backbone of HSA to form pPB-HSA [24]. During liver fibrosis, PDGF is the major cytokine involved in the proliferation of HSC [26,27] and the PDGF-β receptor is highly upregulated on the cell membrane of activated HSC.

In **chapter 7** the two different HSC-selective carriers coupled to 15d-PGJ_2 were investigated. Both conjugates showed specific binding to HSC *in vitro*. However, a selective antagonist for scavenger receptors, reduced the binding of 15d-PGJ_2-M6P-HSA to HSC, revealing the involvement of scavenger receptors in the cellular uptake. The effect of the scavenger receptor antagonist was not detected with 15d-PGJ_2-pPB-HSA, indicating that this conjugate is not interacting with scavenger receptors on HSC. We showed that both conjugates rapidly accumulated in the fibrotic liver *in vivo*. The intrahepatic distribution revealed that 15d-PGJ_2-M6P-HSA was mainly taken up by both HSC and Kupffer cells. In contrast, 15d-PGJ_2-pPB-HSA accumulated more selectively in HSC with the Kupffer cell being less involved in the liver uptake.

Normal liver sections displayed a parenchymal expression of the IGF-II/M6P-receptor, whereas cirrhotic livers clearly showed an increased expression of the IGF-II/M6P-receptors in the non-parenchymal cells. The PDGF-β receptor expression in normal human livers was mainly found along the portal and central blood vessels. Cirrhotic human livers showed a massive increase in PDGF-β receptor expression, mainly in the fibrous bands around the portal areas. Literature data indicate that the activated myofibroblasts are responsible for this increased expression [26].

We concluded that both carriers differ with respect to receptor specificity *in vitro*, leading to differences in intrahepatic distribution during fibrosis. Nevertheless, both carriers seem applicable for the purpose of the delivery of drugs to the most pivotal cells during fibrosis, although each carrier has its own advantage and disadvantage.

GENERAL DISCUSSION

The growing knowledge on the regulation of apoptosis in normal tissue and, even more relevant, on the deregulation during several forms of diseases (i.e. several forms of cancer) has resulted in the introduction of apoptosis-inducing drugs. These drugs specifically target the apoptosis regulatory pathways. Some of these drugs already entered clinical trials and may eventually alter current cancer therapy treatment [28]. Liver fibrosis, associated with loss of the control over the tissue repair process due to excessive proliferation of activated HSC, is also a disease in which apoptosis-inducing drugs might be beneficial. However, unrestricted apoptosis should not occur or at least should be minimized. We therefore designed a HSC-specific apoptosis-inducing drug conjugate and tested this conjugate *in vitro* and *in vivo*. Although we found an induction of apoptosis *in vitro* in the target cells, we were not able to find a significant apoptotic response *in vivo*. An explanation for this may be that apoptosis occurred very fast, ranging from minutes to a few hours after treatment [29], whereas we examined the effects on the fibrotic process after 24 hours. Moreover, it has been shown that apoptotic cells are rapidly and efficiently removed by macrophages [30] resulting in a short half-life and a narrow window for detection of the apoptotic bodies [31]. However, we did find significant beneficial effects of the targeted drugs in the fibrotic rats: there were less HSC and a reduced hepatic collagen synthesis.

In this thesis, the Bile Duct Ligation (BDL) model in rats [32] was used to study the antifibrotic effect of targeted apoptosis-inducing drugs. Initially, we chose this model because the fibrotic process in this model is rapid, though and highly reproducible. However, the ongoing fibrotic response induced by the accumulated bile in the bile ducts results in a pathological process in the portal area. The conjugates that we prepared predominantly accumulate in the central to portal zone of the liver. In addition, the effect of the targeted therapy is not directed at the cholestasic process, but rather against the activated hepatic stellate cell, which is a secondary response to the accumulated bile. Only a biliojejunal anastomis of these fibrotic BDL livers can result in a complete resolution of fibrosis [33,34]. Another animal model for liver fibrosis, in which the fibrotic response occurs in the central to portal area of the liver, would be more appropriate to test our drug delivery conjugates. In

this respect, the CCl_4 model for liver fibrosis could be a suitable model to study the effect of drug delivery to activated HSC. Another advantage of the latter model is that the hepatic injury that leads to liver fibrosis, can be terminated, which opens the option to study an improvement of the resolution process of liver fibrosis. This CCl_4 model is inducible in both rats and mice, and especially the mouse CCl_4 model would create an advantage for targeted therapy studies since less conjugate (prepared in a milligram scale in our lab) will be required for these studies.

FUTURE PERSPECTIVES

This thesis shows the feasibility of drug targeting to the most relevant cell during the progression of liver fibrosis; the HSC. Two types of carriers have been explored (M6P-HSA and pPB-HSA) and both can be considered as promising. Each carrier has its own advantages and disadvantages.

The M6P-HSA carrier has already been tested in several *in vivo* studies exploring the effect of HSC-targeted drugs. Next to 15d-PGJ_2 and gliotoxin, also losartan, gleevec, pentoxyfilline, doxorubicin and mycophenolic acid have been selectively delivered to the HSC during fibrosis. In all these cases, significant pharmacological effects in bile duct ligated rats have been found. However, in none of these studies, the fibrotic process was fully resolved. Consequently, much effort has yet to be undertaken to increase the efficacy of this targeted treatment [35-40].

The PDGF-β receptor is involved in fibrotic diseases in a variety of organs including lung, kidney, liver, skin and intestine. As a consequence, this receptor is increased on the myofibroblastic cells during all these different fibrotic diseases [26,27,41-43]. Therefore, the pPB-HSA carrier might serve as a cell-selective carrier not only for liver fibrosis, but for all kinds of fibrotic diseases in which the PDGF-β receptor plays a pivotal role during the fibrotic process.

In general, drugs should contain appropriate functional groups for covalent conjugation. Recent developments in drug-linker technologies resulted in a platinum-based linker which allows the coupling of drugs via their aromatic nitrogens. Such groups are present in the chemical structure of many drug molecules. This universal linkage system (ULS) can therefore conjugate drug molecules which can not be coupled based on the common drug coupling reactions [39]. This ULS-based linker technology therefore largely broadens the spectrum of drugs to be coupled to a carrier.

Previously, liposomes have been used to encapsulate prostacycline and prostaglandin E_1 to prolong their circulation time and avoid their side effects [44]. These liposome-prostaglandins accumulated in the vascular lesions of hypersensitive rats [45]. Recently, liposomes have been designed that have M6P-HSA coupled to the

liposomal surface. These M6P-HSA liposomes were taken up by HSC *in vitro* and *in vivo* [46]. Encapsulation of 15d-PGJ_2 in these M6P-HSA liposomes can potentially leads to a selective delivery to activated HSC.

The targeting techniques presented in this thesis may therefore be applicable for a broad spectrum of drugs for different fibrotic diseases and various types of drug carrier systems can be employed.

CONCLUSION

The work presented in this thesis describes the targeting of apoptosis-inducing drugs to the HSC in fibrotic livers. The GTX and 15d-PGJ_2 HSC-selective conjugates that have been prepared, show rapid and complete accumulation in the fibrotic liver. Treatment of rats with these conjugates during liver fibrosis clearly induced pharmacologic effects on key parameters of fibrosis. It can be concluded that the chosen targeted approach is feasible for apoptosis-inducing drugs and may provide a relevant step in the design of a new treatment of this chronic liver disease.

REFERENCE LIST

1. Friedman S L. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. J Biol Chem 2000; 275(4):2247-2250.
2. Lotersztajn S, Julien B, Teixeira-Clerc F, Grenard P, Mallat A. Hepatic fibrosis: Molecular mechanisms and drug targets. Annual Review of Pharmacology and Toxicology 2005; 45:605-628.
3. Iredale JP, Benyon RC, Pickering J, McCullen M, Northrop M, Pawley S, Hovell C, Arthur MJP. Mechanisms of spontaneous resolution of rat liver fibrosis. Hepatic stellate cell apoptosis and reduced hepatic expression of metalloproteinase inhibitors. J Clin Invest 1998; 102(3):538-549.
4. Issa R, Zhou X, Constandinou CM, Fallowfield J, Millward-Sadler H, Gaca MDA, Sands E, Suliman I, Trim N, Knorr A, Arthur MJP, Benyon RC, Iredale JP. Spontaneous recovery from micronodular cirrhosis: Evidence for incomplete resolution associated with matrix cross-linking. Gastro 2004; 126:1795-1808.
5. Elsharkawy AM, Oakley F, Mann DA. The role and regulation of hepatic stellate cell apoptosis in reversal of liver fibrosis. Apoptosis 2005; 10(5):927-939.
6. Canbay A, Friedman S L, Gores GJ. Apoptosis: the nexus of liver injury and fibrosis. Hepatology 2004; 39(2):273-278.
7. Wright MC, Issa R, Smart DE, Trim N, Murray GI, Primrose JN, Arthur MJP, Iredale JP, Mann DA. Gliotoxin stimulates the apoptosis of human and rat hepatic stellate cells and enhances the resolution of liver fibrosis in rats. Gastro 2001; 121:685-698.
8. Dekel R, Zvibel I, Brill S, Brazovsky E, Halpern Z, Oren R. Gliotoxin ameliorates development of fibrosis and cirrhosis in a thioacetamide rat model. digestive diseases and sciences 2003; 48(8):1642-1647.

9. Beljaars L, Molema G, Weert B, Bonnema H, Olinga P, Groothuis GMM, Meijer DKF, Poelstra K. Albumin modified with mannose 6-phosphate: A potential carrier for selective delivery of antifibrotic drugs to rat and human hepatic stellate cells. Hepatology 1999; 29(5):1486-1493.
10. Beljaars L, Olinga P, Molema G, De Bleser P, Geerts A, Groothuis GMM, Meijer DKF, Poelstra K. Characteristics of the hepatic stellate cell-selective carrier mannose 6-phosphate modified albumin (M6P(28)-HSA). Liver 2001; 21(5):320-328.
11. Moss DW, Henderson AR. Clinical enzymology. In: Burtis CA, Ashwood ER, editors. Tietz textbook of clinical chemistry. Philadelphia: Saunders, 1999: 788-797.
12. Nanji AA. Role of Kupffer cells in alcoholic hepatitis. Alcohol 2002; 27:13-15.
13. Gallois C, Habib A, Tao J, Moulin S, Maclouf J, Mallat A, Lotersztajn S. Role of NF-kB in the antiproliferative effect of endothelin-1 and tumor necrosis factor-alpha in human hepatic stellate cells. Involvement of cyclooxygenase-2. J Biol Chem 1998; 273(36):23183-23190.
14. Mallat A, Gallois C, Tao JC, Habib A, Maclouf J, Mavier P, Préaux A-M, Lotersztajn S. Platelet-derived growth factor-BB and thrombin generate positive and negative signals for human hepatic stellate cell proliferation. Role of a prostaglandin/cyclic AMP pathway and cross-talk with endothelin receptors. J Biol Chem 1998; 273(42):27300-27305.
15. Schade S, Bezugla Y, Kolada A, Kamionka S, Scheibe R, Dieter P. Diverse functional coupling of cyclooxygenase 1 and 2 with final prostanoid synthases in liver macrophages. Biochem Pharmacol 2002; 64:1227-1232.
16. Li LY, Tao JC, Davaille J, Feral C, Mallat A, Rieusset J, Vidal H, Lotersztajn S. 15-deoxy-Delta(12,14)-prostaglandin J2 induces apoptosis of human hepatic myofibroblasts - A pathway involving oxidative stress independently of peroxisome-proliferator-activated receptors. J Biol Chem 2001; 276(41):38152-38158.
17. Cuzzocrea S, Wayman NS, Mazzon E, Dugo L, Di Paola R, Serraino I, Britti D, Chatterjee PK, Caputi AP, Thiemermann C. The cyclopentenone prostaglandin 15-deoxy-Delta(12,14)-prostaglandin J(2) attenuates the development of acute and chronic inflammation. Molecular Pharmacology 2002; 61(5):997-1007.
18. Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. Nature 1998; 391(6662):79-82.
19. Li LY, Julien B, Grenard P, Teixeira-Clerc F, Mallat A, Lotersztajn S. Molecular mechanisms regulating the antifibrogenic protein heme-oxygenase-1 in human hepatic myofibroblasts. Journal of hepatology 2004; 41(3):407-413.
20. Li LY, Grenard P, Van Nhieu JT, Julien B, Mallat A, Habib A, Lotersztajn S. Heme oxygenase-1 is an antifibrogenic protein in human hepatic myofibroblasts. Gastro 2003; 125(2):460-468.
21. Miyahara T, Schrum L, Rippe R, Xiong SG, Yee HF, Motomura K, Anania FA, Willson TM, Tsukamoto H. Peroxisome proliferator-activated receptors and hepatic stellate cell activation. J Biol Chem 2000; 275(46):35715-35722.
22. Straus DS, Glass CK. Cyclopentenone prostaglandins: New insights on biological activities and cellular targets. Medicinal Research Reviews 2001; 21(3):185-210.
23. Beljaars L, Molema G, Schuppan D, Geerts A, De Bleser PJ, Weert B, Meijer DKF, Poelstra K. Successful targeting to rat hepatic stellate cells using albumin modified with cyclic peptides that recognize the collagen type VI receptor. J Biol Chem 2000; 275(17):12743-12751.
24. Beljaars L, Weert B, Geerts A, Meijer DKF, Poelstra K. The preferential homing of a platelet derived growth factor receptor-recognizing macromolecule to fibroblast-like cells in fibrotic tissue. Biochemical Pharmacology 2003; 66(7):1307-1317.

25. De Bleser PJ, Jannes P, Vanbuuloffers SC, Hoogerbrugge CM, Vanschravendijk CFH, Niki T, Rogiers V, Vandenbrande JL, Wisse E, Geerts A. Insulin-Like Growth Factor-Ii Mannose 6-Phosphate Receptor Is Expressed on Ccl4-Exposed Rat Fat-Storing Cells and Facilitates Activation of Latent Transforming Growth-Factor-Beta in Cocultures with Sinusoidal Endothelial-Cells. Hepatology 1995; 21(5):1429-1437.
26. Pinzani M, Milani S, Herbst H, DeFranco R, Grappone C, Gentilini A, Caligiuri A, Pellegrini G, Ngo DV, Romanelli RG, Gentilini P. Expression of platelet-derived growth factor and its receptors in normal human liver and during active hepatic fibrogenesis. American Journal of Pathology 1996; 148(3):785-800.
27. Wong L, Yamasaki G, Johnson RJ, Friedman SL. Induction of beta-platelet-derived growth factor receptor in rat hepatic lipocytes during cellular activation in vivo and in culture. J Clin Invest 1994; 94(4):1563-1569.
28. Fesik SW. Promoting apoptosis as a strategy for cancer drug discovery. Nat Rev Cancer 2005; 5(11):876-885.
29. Rust C, Gores GJ. Apoptosis and liver disease. American Journal of Medicine 2000; 108(7):567-574.
30. Maderna P, Godson C. Phagocytosis of apoptotic cells and the resolution of inflammation. Biochim Biophys Acta 2003; 1639(3):141-151.
31. Cummings J, Ward TH, Ranson M, Dive C. Apoptosis pathway-targeted drugs - from the bench to the clinic. Biochimica et Biophysica Acta-Reviews on Cancer 2004; 1705(1):53-66.
32. Kountouras J, Billing BH, Scheuer PJ. Prolonged bile duct obstruction: a new experimental model for cirrhosis in the rat. Br J Exp Pathol 1984; 65(3):305-311.
33. Issa R, Williams E, Trim N, Kendall T, Arthur MJP, Reichen J, Benyon RC, Iredale JP. Apoptosis of hepatic stellate cells: involvement in resolution of biliary fibrosis and regulation by soluble growth factors. Gut 2001; 48(4):548-557.
34. Abdelaziz G, Lebeau G, Rescan PY, Clement B, Rissel M, Deugnier Y, Campion JP, Guillouzo A. Reversibility of Hepatic-Fibrosis in Experimentally Induced Cholestasis in Rat. American Journal of Pathology 1990; 137(6):1333-1342.
35. Greupink R, Bakker HI, Reker-Smit C, van Loenen-Weemaes AM, Kok RJ, Meijer DKF, Beljaars L, Poelstra K. Studies on the targeted delivery of the antifibrogenic compound mycophenolic acid to the hepatic stellate cell. Journal of hepatology 2005; 43(5):884-892.
36. Gonzalo T, Poelstra K, Beljaars L, Keri G, Orfi L, Kok RJ. Specific targeting of a PDGFR tyrosine kinase inhibitor to activated hepatic stellate cells as a promising technology to reduce liver fibrosis. Hepatology 2005; 42(4):733A.
37. Gonzalo T, Bataller R, Sancho-Bru P, Fontdevilla C, Swart J, Beljaars L, Arroyo V, Poelstra K, Gines P, Kok RJ. Short-term treatment with losartan targeted to activated stellate cells reduces advanced liver fibrogenesis: A new strategy to treat liver fibrosis. Hepatology 2005; 42(4):604A.
38. Hagens WI, Beljaars L, Julien B, de Jager-Krikken A, Reker-Smit C, van Loenen-Weemaes A, de Vries W, Lotersztajn S, Poelstra K. Anti-fibrotic effects of targeted 15D-prostaglandin J2 in human hepatic myofibroblasts and in fibrotic rats. Hepatology 2005; 42(4):598A.
39. Gonzalo T, Talman EG, van d, V, Temming K, Greupink R, Beljaars L, Reker-Smit C, Meijer DK, Molema G, Poelstra K, Kok RJ. Selective targeting of pentoxifylline to hepatic stellate cells using a novel platinum-based linker technology. J Control Release 2006; 111(1-2):193-203.
40. Greupink R, Bakker H, de Ruijter M, van Loenen-Weemaes AM, Reker-Smit C, Meijer DK, Beljaars L, Poelstra K. Specific delivery of antiproliferative drugs to the hepatic stellate cell; Effects of targeted doxorubicin in bile duct ligated rats. Hepatology 2004; 40(4):544A-545A.

41. Alpers CE, Seifert RA, Hudkins KL, Johnson RJ, Bowen-Pope DF. Developmental patterns of PDGF B-chain, PDGF-receptor, and alpha-actin expression in human glomerulogenesis. Kidney Int 1992; 42(2):390-399.
42. Bonner JC. Regulation of PDGF and its receptors in fibrotic diseases. Cytokine Growth Factor Rev 2004; 15(4):255-273.
43. Klareskog L, Gustafsson R, Scheynius A, Hallgren R. Increased expression of platelet-derived growth factor type B receptors in the skin of patients with systemic sclerosis. Arthritis Rheum 1990; 33(10):1534-1541.
44. Mizushima Y. Lipo-Prostaglandin Preparations. Prostaglandins Leukotrienes and Essential Fatty Acids 1991; 42(1):1-6.
45. Mizushima Y, Hamano T, Haramoto S, Kiyokawa S, Yanagawa A, Nakura K, Shintome M, Watanabe M. Distribution of Lipid Microspheres Incorporating Prostaglandin-E1 to Vascular-Lesions. Prostaglandins Leukotrienes and Essential Fatty Acids 1990; 41(4):269-272.
46. Adrian JE, Poelstra K, Scherphof GL, Molema G, Meijer DK, Reker-Smit C, Morselt HW, Kamps JA. Interaction of targeted liposomes with primary cultured hepatic stellate cells: Involvement of multiple receptor systems. J Hepatol 2006; 44(3):560-567.